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TYPES OF ORGANISMS SURVIVING IN COMMERCIALLY PASTEURIZED CITRUS JUICES IN FLORIDA[1]

by
Arthur J. Nolte and Harry W. von Loesecke
U. S. Citrus Products Station, Bureau of Agricultural Chemistry and Engineering
U. S. Department of Agriculture, Winter Haven, Florida

Although citrus juices (primarily grapefruit) have been canned in Florida for many years by exhausting, sealing, and then processing, this method is now being replaced by the more modern technique of high-short[2] pasteurization. Whereas the old method subjected the juice to temperatures of 80 to 85° C. (176 to 185° F.) for periods ranging from one-half to one hour, in the high-short procedure the juice is heated at 85 to 88° C. (185 to 190° F.) for only a few seconds. It has been found that this method, especially when used in conjunction with efficient de-aeration, aids in preserving the natural aroma and taste of the juice and its vitamin C potency and prolongs storage life. These temperatures and times, too, are probably sufficient to inactivate enzymes responsible for subsequent settling or "curdling" of the processed juice.

A bacteriological study of organisms present in fresh citrus juices and those surviving high-short pasteurization should be of definite practical value. Pathogenic organisms of the colon-typhoid-dysentery group are said to be found on citrus fruits and in their juices, Garcia (1911). Possible sources of such contamination may include flies, picking and handling, polluted wash waters, and the use of drops. These pathogens and indicators of pathogens would not survive pasteurization temperatures in the case of citrus juices because of the acidity of the medium. For this reason, microorganisms in citrus juices are more easily destroyed by high-short pasteurization than microorganisms in milk, Breed (1932).

According to Berry (1932), thermophilic and similar heat-resistant organisms have been found in canned tomato juice, giving the juice an off-flavor which was almost nauseating in extreme cases. In the present work thermophiles were isolated from pasteurized citrus juices. These organisms were plated out on dextrose agar and incubated at 55° C.(131° F.) under aerobic and anaerobic conditions.

EXPERIMENTAL PROCEDURE

Samples of juice from the same lots before and after processing were obtained at different citrus canneries. Fresh juice was sampled immediately as it came from the extractors or was taken from blending tanks. The pasteurized juice was obtained either from the filler bowl or from sealed cans after they had passed through the cooling tanks. The samples were plated within 30 minutes after they had been obtained.

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2 This is the term suggested by Shrader as a substitute for "flash". This latter term is considered a misnomer by most food technologists.

Plating was carried out on nutrient agar, wort agar, trypsin-digest agar, and tomato-juice agar. For organisms of the coli-aerogenes group, lactose broth buffered with potassium acid phosphate was used and incubated according to the methods of the American Public Health Association (1933). Results from these tests were confirmed by Endo's agar and Brilliant Green Lactose Bile Agar with reinoculation of picked colonies from Endo's agar into Lactose broth. With the exception of wort-agar plates, incubation was at 37°C. (98.6°F.) for 72 hours. Wort-agar plates were incubated at 25°C. (77°F.) for 72 hours; and some of the counts on nutrient agar were made at 20°C. (68°F.). Anaerobic counts were accomplished according to Dick's anaerobic plate method, used by Tanner (1928), in which dilutions were plated as usual, an additional layer of agar being poured over the hardened agar in the plate. After cooling, sterile melted paraffin was poured on this second coating of agar. Plates were then placed in a Novy jar and the jar exhausted.

DISCUSSION OF RESULTS

All of the raw-juice samples showed yeasts, molds, and bacteria in considerable numbers (Tables 1,2,3,4,5,and 6). Variations in counts could not be correlated with the apparent cleanliness of the plant, nor was there any significant difference in the counts of samples taken periodically during the season. Camp and Stahl (1934) in one of their papers, report a considerably lower count for freshly extracted orange juice than was obtained by the authors. Their juice was prepared in the laboratory, but without special precautions as to sterility of equipment. It would seem that the counts reported here for samples representing commercial extraction of citrus juices are unnecessarily high and undoubtedly could be lowered by the use of chlorine after the fruit has passed through the washers.

In only one sample of pasteurized grapefruit juice was a mold count obtained. This sample was taken from a can collected at the start of the day's run at one plant.

A number of colonies of yeasts and molds from raw juice were obtained from the wort-agar plates for subcultures. These were examined and identified as follows:

Saccharomyces cerevisiae
Saccharomyces apiculatus
Torula (one a pink torula) sp.
Dematium sp.
Monila sp.
Aspergillus glaucus
Saccharomyces ellipsoideus
Saccharomyces pastorianus
Mycoderma cerevisiae
Oidium (one a red oidium) sp.
Penicillium glaucum
Aspergillus sp.

Over 100 cultures of bacteria were isolated from the raw juice. No attempt was made to identify the organisms in the raw juice because it was felt the time involved to carry out a complete study would not be justified. It was considered more important to attempt the identification of those organisms surviving the high-short pasteurization.

None of the bacteria isolated from pasteurized juice would grow on orange or grapefruit juice agars under either aerobic or anaerobic conditions at 25,37, and 55°C. (77,98.6 and 131°F.). Some of the isolated organisms did, however, show growth on dextrose agar at 55°C. The inability of all the organisms isolated in our study, including the thermophiles, to grow in citrus juices indicates

that the organisms are not a factor in the development of off-flavors in canned juice. The molds isolated, however, did grow under aerobic conditions at 25°C.

Survival and destruction determinations of organisms isolated from pasteurized juice were made by inoculating sterile orange juice (pH 3.7) with a loop of a six-day culture of the organism. The inoculated juice was contained in Pyrex test tubes which were placed in a water bath heated to 85°C. (185°F.). A similar tube of orange juice with a thermometer was used as control for temperature readings. One minute and two minutes after the juice had reached the desired temperature a loop was removed from the inoculated tube and streaked on nutrient agar. At intervals of two minutes additional loops of material were withdrawn. A temperature of 85°C. was selected as it is the minimum temperature of pasteurization usually employed in Florida citrus canneries. The other temperature used in these tests was 99°C.(210.2°F.). Although the tubes were in a boiling water bath, the contents never actually reached 100°C. (212°F.).

Survival and destruction times, determined as described above, of unidentified organisms from pasteurized citrus juices are given (Table 7).

The destruction time of most of the organisms surviving high-short pasteurization is sufficiently long to prevent their destruction by this method of pasteurization. Their inability to grow in orange or grapefruit juice, however, renders them unable to cause spoilage from a biological point of view.

Organisms isolated from pasteurized juice and identified consisted of the following[3]: Bacillus mesentericus, Bacillus subtilis, Bacillus vulgatus, Mucor sp. and Penicillium glaucum (these two molds were isolated from one of the first cans from the line when the day's run was started), Bacillus cylindricus (or Bacillus cytaseus), Lactabacillus thermophilus, Bacillus non diastaticus, Bacillus cereus, and Sarcina sp.

SUMMARY

Samples of fresh and high-short commercially pasteurized citrus juices (orange and grapefruit) were taken at intervals during the canning season from five different canneries for bacteriological examination.

The yeast and mold count of raw juice ranged from a minimum of 800 to a maximum of 514,600 per cc. Bacterial count (aerobic) ranged from a minimum of 800 to a maximum of 744,00 per cc.

Variation in counts could not be correlated with the apparent cleanliness of the plant, nor was there any significant difference in counts of samples taken periodically during the season.

Coli-aerogenes group of organisms showed a count ranging from a minimum of zero to a maximum of 1,000 per cc in raw juice.

Escherichia coli was shown to be present in raw juice but absent in pasteurized juice.

3 Identified and named according to Bergey's "Mannal of Determinative Bacteriology.", 4th Ed. (1934).

Water from washing tanks in the two canneries sampled showed high coli-aerogenes content.

Bacterial count of pasteurized juice showed a minimum of zero and a maximum of 30 per cc under aerobic conditions; and a minimum of zero to a maximum of 42 per cc under anaerobic conditions. None of the bacteria isolated from the pasteurized juice would grow on orange or grapefruit agars either aerobically or anaerobically. Some, however, grew on dextrose agar at 55°C.(131°F.).

Survival and destruction times of most of the organisms not destroyed by high-short pasteurization were sufficiently high to prevent their destruction by this means of pasteurization. Their inability to grow in orange or grapefruit juice, however, renders them unable to cause spoilage from a biological point of view.

High-short pasteurization of orange and grapefruit juice as practiced in the canneries studied is shown to be efficient in destroying organisms which may later cause biological spoilage.

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Table 1 - Bacteriological Examination of Raw & Pasteurized Canned Cit.Juice-Plant A (Organisms per c.c.)

Sampling Date	Wort agar	Nutrient agar: Aerobes 37°C. (98.6°F)	Nutrient agar: Anaerobes 37°C.	Nutrient agar: Aerobes 20°C. (68°F)	Nutrient agar: Anaerobes 20°C.	Tomato-J. agar: Aerobes 37°C.	Tomato-J. agar: Anaerobes 37°C.	Trypsin-digest agar: Aerobes 37°C.	Trypsin-digest agar: Anaerobes 37°C.
RAW JUICE									
1/7/38	119,700	87,000	-----	58,900	-----	------	-------	-----	------
1/17/38	1,900	1,800	-----	2,500	-----	------	-------	-----	------
2/4/38	13,000	39,700	-----	10,000	-----	------	-------	-----	------
2/19/38	44,000	37,000	-----	44,000	-----	47,000	-------	11,000	------
3/10/38	354,000	438,000	-----	180,000	-----	744000	-------	657000	------
4/15/38	514,600	106,000	91000	217,800	[illegible]	371000	272,800	316000	208,000
Pasteurized Canned Juice									
1/7/38	0	0	-----	0	-----	----	-----	----	-----
1/17/38	0	0	-----	0	-----	----	-----	----	-----
2/4/38	0	0	-----	0	-----	----	-----	----	-----
2/19/38	0	0	-----	0	-----	0	-----	0	-----
3/10/38	5	1	-----	2	-----	0	-----	0	-----
4/15/38	0	0	0	0	0	0	0	1	1

Table 2-Bacteriological Examination of Raw & Pasteurized Canned Citrus Juice.--
Plant B[1] (Organisms per cc)

Sampling date	Wort Agar	Nutrient Agar 37° C. (98.6°F)	Nutrient Agar 20° C. (68°F)	Tomato J. agar 37° C.	Trypsin digest agar 37° C.
RAW JUICE					
1/7/38	18,600	2,000	25,000	-----	-----
1/17/38	800	800	3,400	-----	-----
2/4/38	17,000	13,000	48,100	-----	-----
2/19/38	22,000	31,000	29,000	28,000	16,000
3/17/38	14,000	6,000	35,000	2,000	9,000
PASTEURIZED CANNED JUICE					
1/7/38	0	11	2	-----	-----
1/17/38	0	0	0	-----	-----
2/4/38	0	0	0	-----	-----
2/19/38	0	0	20	1	0
3/17/38	0	0	0	0	0

1 Counts are all aerobic.

Table 3-Bacteriological Examination of Raw and Pasteurized Canned Citrus Juice --
Plant C (Organisms per cc)

Sampling date	Wort Agar	Nutrient Agar Aerobes 37°C. (98.6°F.)	Nutrient Agar Anaerobes 37°C.	Nutrient Agar Aerobes 20°C. (68°F.)	Nutrient Agar Anaerobes 20°C.	Tomato-J agar Aerobes 37°C.	Tomato-J agar Anaerobes 37°C.	Trypsin digest agar Aerobes 37°C.	Trypsin digest agar Anaerobes 37°C.
Raw Juice									
1/7/38	44,000	40,100	---	62,000	---	---	---	---	---
1/17/38	11,600	14,300	---	15,000	---	---	---	---	---
2/4/38	121,000	89,000	---	136,400	---	---	---	---	---
2/19/38	92,000	106,000	---	236,000	---	125000	---	98,000	---
3/10/38	87,500	38,000	---	26,000	---	58000	---	37,000	---
3/24/38	123,500	73,000	---	160,000	---	76000	---	130,000	---
4/13/38	---	----	11,160	---	31620	---	72540	---	37,450
4/28/38	59,000	58,000	16,000	85,000	75000	80000	85000	15,500	84,500
PASTEURIZED CANNED JUICE									
1/7/38	0	0	-	0	-	-	-	-	-
1/17/38	0	0	-	0	-	-	-	-	-
2/4/38	0	5	-	0	-	-	-	-	-
2/10/38	0	0	-	0	-	0	-	1	-
3/10/38	0	0	-	0	-	0	-	1	-
3/24/38	0	-	-	0	-	0	-	0	-
4/13/38	0	-	0	-	1	-	0	-	0
4/28/38	0	0	0	0	0	0	0	0	0

Table 4-Bacteriological Examination of Raw and Pasteurized Canned Citrus Juice --
Plant D (Organisms per cc)

Sampling date	Wort Agar	Nutrient agar Aerobes 37° C. (98.6°F)	Nutrient agar Anaerobes 37°C.	Nutrient agar Aerobes 20° C. (68°F.)	Nutrient agar Anaerobes 20°C.	Tom.J agar Aerobes 37° C.	Tom.J agar Anaerobes 37°C.	Tryp. dig. agar Aerobes 37°C.	Tryp. dig. agar Anaerobes 37°C.
Raw j., 5/5/38	165000	56000	53000	102000	35000	117000	109000	167000	79000
Can.j., 5/5/38	0	6	42	30	18	2	3	7	4

Table 5 - Bacteriological Examination of Raw and Pasteurized Canned Citrus Juice - Plant E (Organisms per cc.)

Sampling date	Wort agar	RAW JUICE							
		Nutrient Agar				Tomato-J. Agar		Trypsin-digest Agar	
		Aerobes 37°C. (98.6°F)	Anaerobes 37°C.	Aerobes 20°C. (68°F.)	Anaerobes 20°C.	Aerobes 37°C.	Anaerobes 37°C.	Aerobes 37°C.	Anaerobes 37°C.
4/15/38	32,000	26,000	25,000	155,000	110,000	21000	18000	25,000	15,000
5/28/38	24,000	21,000	13,000	36,000	5,000	77000	34000	42,000	55,000
		PASTEURIZED JUICE							
4/15/38	0	19	5	11	6	3	2	5	2
5/28/38	0	1	3	1	0	1	0	0	0

Table 6 - Presumptive Coli-Aerogenes Lactose-Broth Tests of Raw Citrus Juice and Water from Fruit-Washing Tanks[1] (Organisms per cc)

Sampling Date	Plant A[2]	Plant B	Plant C[3]
1/7/38	<1	<1	0
1/17/38	<1	<1	<1
2/4/38	1,000	1,000	0
2/17/38	100	100	55
3/17/38	1	0	1

1 Quantitative counts computed according to "Standard Methods of Water Analysis". 7th ed. p. 132(b) (1933).
2 Fruit-washing tank water, Plant A - 10,000 on 2/17/38.
3 Fruit-washing tank water, Plant C - 1,000 on 2/4/38.

Table 7 - Survival and Destruction in Orange Juice (pH 3.7) of Organisms (Unidentified) Isolated from Pasteurized Citrus Juices[1]

Organism No.	Survival and destruction times
1, 15, 21, 23	Survived 10 min. at 99°C. (210.2°F.)
4	Destroyed in 7 min. at 99°C.
8, 19, 14	Destroyed in 5 min. at 85°C. (185°F.)
10	Destroyed in 1 min. at 99°C.
16	Destroyed in 3 min. at 85°C.

1 See text for method of determination.

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